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A breathtaking feat

To compete with the gut microbiota, salmonella drives its host to provide a respiratory electron acceptor

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Salmonella is a common cause of food poisoning. However, after ingestion the pathogen has to compete with resident microbes that already occupy the intestinal lumen (microbiota), which poses a challenge for Salmonella to successfully colonize this niche. Recent data show that Salmonella elicits help from the host immune response to beat the competition. After arriving in the intestine, Salmonella elicits acute intestinal inflammation. The respiratory burst of neutrophils that transmigrate into the intestinal lumen during inflammation oxidizes endogenous sulfur compounds to generate a respiratory electron acceptor, tetrathionate. As a result, Salmonella can use tetrathionate respiration to outgrow the fermenting microbiota in the anaerobic environment of the gut, which promotes transmission of the pathogen. This principle might be used by other gut microbes and contribute to changes in the microbiota composition observed during inflammation.

Key words: gastroenteritis, salmonella, inflammation, microbiota, tetrathionate

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The human intestine is host to a diverse community of microbes comprised of more than 160 bacterial species.[1] In healthy individuals, this bacterial population is dominated by species belonging to the Bacteroidetes and Firmicutes phyla. However, intestinal inflammation is accompanied by changes in the microbiota composition. For example, the microbiota from patients with inflammatory bowel disease shows an increased relative abundance of bacteria belonging to the *Proteobacteria* phylum.[2] The mechanisms responsible for these changes in the microbial community structure remain unknown.

A study of small bowel transplant patients reveals one possible mechanism for shaping the microbiota composition.[3] After the initial surgery, access into the small bowel transplant is maintained by surgical creation of an ileostomy (portal) through the abdominal wall. Analysis of the microbiota in close proximity to the ileostomy reveals an increase in the relative abundance of *Proteobacteria* belonging to the family *Enterobacteriaceae*, presumably because the portal allows oxygen to reach the otherwise anaerobic distal ileum. Consistent with this idea, the microbiota composition returns to normal after surgical closure of the ileostomy. This observation introduced the concept that access to a respiratory electron acceptor in the anaerobic environment of the gut can drive changes in the microbial community structure.

Some enteric pathogens, such as *Citrobacter rodentium*, *Campylobacter jejuni* and *Salmonella enterica* serotype *typhimurium* (*S. typhimurium*), use their virulence factors to trigger acute intestinal inflammation.[4] The ensuing inflammatory response promotes a bloom of these pathogens in the gut, thereby making them a prominent component of the microbiota.[5,6] This microbiota outgrowth is important for transmission of *S. typhimurium* in a mouse model.[7] The mechanism by which inflammation enhances growth of *S. typhimurium* is related to neutrophil transmigration into the intestinal lumen, a hallmark of the disease caused by this pathogen in humans.[8] The respiratory burst of neutrophils in the intestinal lumen oxidizes an endogenous sulfur compound, thiosulfate ($S_2O_3^{2-}$), which originates from the

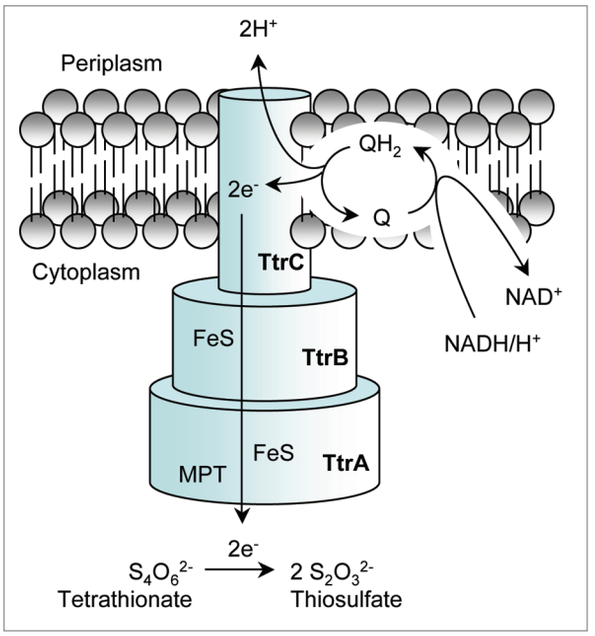

Figure 1. Biochemical model of the tetrathionate reductase in *S. typhimurium*. The tetrathionate reductase in *S. typhimurium* is comprised of the three subunits TtrC, TtrB and TtrA. TtrC is predicted to contain 9 transmembrane helices and belongs to the NrfD-type family (pfam03916). TtrC is likely involved in the transfer of electrons from the quinone (Q) pool to other components of the reductase complex, while at the same time releasing protons (H^+) into the periplasm. TtrB is predicated to contain an iron-sulfur cluster (FeS) binding domain (pfam00037) and may link transport of electrons from TtrC to TtrA, the subunit that actually reduces tetrathionate. TtrA contains a predicted iron-sulfur cluster-binding domain and belongs to the molybdopterin (MPT) binding superfamily (cl09928) which also includes various members of oxidoreductases such as bacterial nitrate reductases, dimethylsulfoxide reductases, thiosulfate reductases and arsenite oxidase. Although molybdopterin co-factor containing enzymes typically catalyses reactions that involve the transfer of an oxygen atom or the cleavage of a C-H bond, molybdopterin is as part of the active center of TtrA directly involved in the reduction of tetrathionate.[18]

detoxification of hydrogen sulfide (H_2S) by enterocytes.[9] The oxidation product of thiosulfate generated during intestinal inflammation is tetrathionate ($S_4O_6^{2-}$),[10] which can serve as a respiratory electron acceptor for *S. typhimurium*[11] (**Fig. 1**). The use of tetrathionate respiration for energy production presents *S. typhimurium* with a significant growth advantage over competing microbes that rely on fermentation.[10] Thus, the generation of a new respiratory electron acceptor as byproduct of the host inflammatory response can drive changes in the microbiota composition in the anaerobic environment of the gut.

Inflammation might promote outgrowth of other microbes through similar mechanisms. The ability of *S. typhimurium* to perform tetrathionate respiration is encoded by five genes in two operons, which form the *ttrSR ttrBCA* gene cluster.[12] The *ttrSR* genes encode a two component regulatory system that controls expression of *ttrBCA*, the genes encoding subunits of the tetrathionate reductase (**Fig. 1**). The *ttrBCA* genes are present in several members of the *Proteobacteria* (**Fig. 2**), including environmental bacteria that may benefit from the presence of tetrathionate in soil or marine sediments.[13] However, a number of *Proteobacteria* might utilize tetrathionate respiration in the host. For example, the genus Citrobacter can use tetrathionate as a terminal electron acceptor[11] and host-mediated inflammation enables *C. rodentium* to increase its relative luminal abundance in the gut.[5] *Proteobacteria* that are able to perform tetrathionate respiration include other pathogens that trigger intestinal inflammation, such as *Yersinia enterocolitica*, *Vibrio parahaemolyticus*, *Vibrio vulnificus* and most Salmonella serotypes (**Fig. 2**).[11] Thus, it seems likely that the generation of tetrathionate during intestinal inflammation provides a luminal growth advantage for these pathogens.

While most Salmonella serotypes can use tetrathionate as a respiratory electron acceptor, this property is absent in a few specialists associated with systemic infections, including *S. enterica* serotypes *paratyphi* A and *gallinarum*.[11] Furthermore, the *ttrSR ttrBCA* gene cluster contains pesudogenes in some *S. enterica* serotype *typhi* isolates.[14] Access to internal organs during systemic infection enables these specialists to utilize altered routes of transmission. For example, chronic carriage in the gallbladder of patients enables *S. typhi* and *S. paratyphi* A to reach the intestine via the bile duct, thereby ensuring transmission by the fecal oral route.[15] Transovarian infection of domestic fowl enables *S. gallinarum* to spread vertically via the egg to the young fowl.[16] It is tempting to speculate that these changes in the route of transmission abolish the need for *S. typhi*, *S. paratyphi* A and *S. gallinarum* to outgrow the microbiota to ensure their transmission to a susceptible host.

Finally, the availability of tetrathionate during intestinal inflammation might help explain changes in the microbiota composition observed during inflammatory bowel disease. For instance, alterations in the composition of the microbiota detected in a mouse model of inflammatory bowel disease include an increased luminal abundance of *Proteus mirabilis*,[17] an organism that is able to perform tetrathionate respiration (**Fig. 2**).[11] Thus tetrathionate respiration might contribute to changes in the microbial community structure observed during intestinal inflammation.

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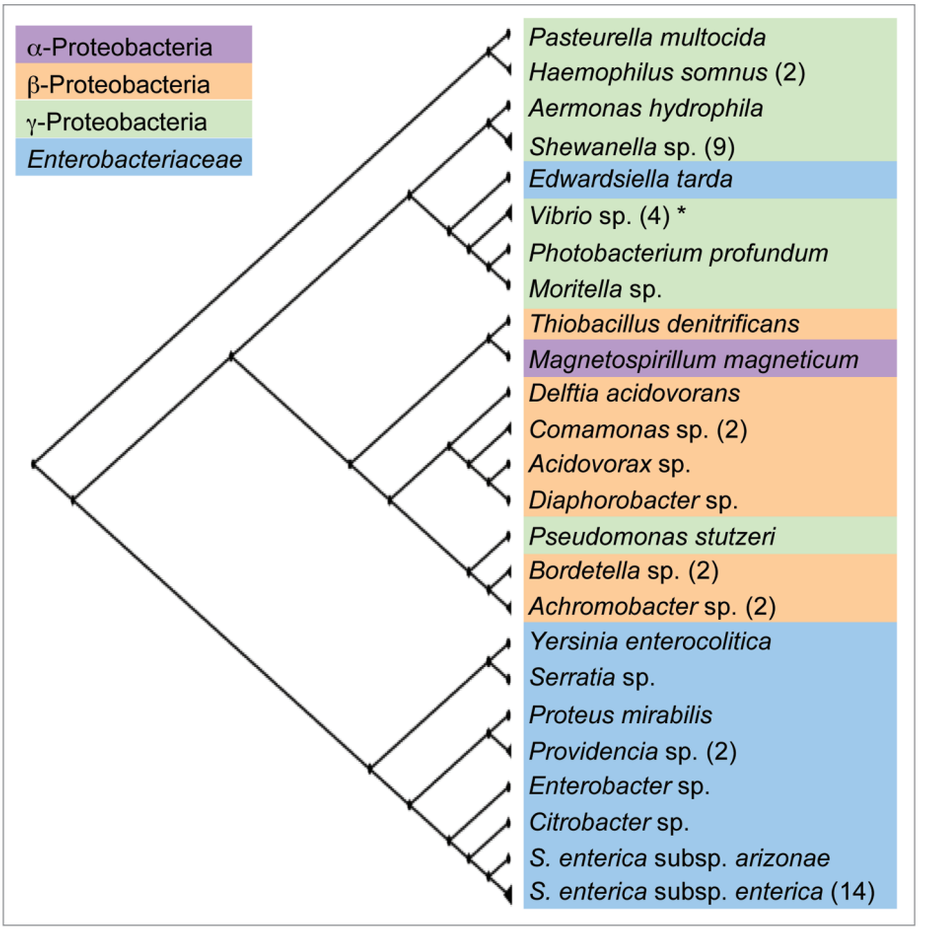

Figure 2. Phylogenetic distribution of tetrathionase reductase subunit A (TtrA). Dendrogram generated by comparing amino acid sequences of TtrA. The number of species within each genus is indicated in brackets. In the case of *Samonella enterica* subsp. *enterica*, the number in brackets represents the number of sequenced serotypes encoding a TtrA protein. Taxonomic affiliation of each bacterial genus to the classes Alpha-*proteobacteria* (purple), Beta-*proteobacteria* (orange), Gamma-*proteobacteria* (green) or the family *Enterobacteriaceae* (blue) is illustrated. *all species were non-cholerae Vibrios. The Basic Local Alignment Search Tool (BLAST) algorithm BLASTP 2.2.24+,[19] was used to identify protein sequences deposited in the GenBank (National Center for Biotechnology Information), the Protein Data Bank (PDB), Swiss Prot (Swiss Institute for Bioinformatics/European Bioinformatics Institute), the Protein Information Resource (PIR), and the Protein Research Foundation Sequence Database (PRF) that share sequence similarity to the TtrA protein (NP_460348.1) of the *S. typhimurium* strain LT2. An unrooted tree using the neighbor joining method,[20] was generated from protein sequences with more than 55% identity.

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